Paramylon synthesis by *Euglena gracilis* photoheterotrophically grown under low O$_2$ pressure

**Description of a mitochloroplast complex**

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**Abstract.** Special culture conditions for *Euglena gracilis* Z and ZR are described. They induce interactions between the chloroplast and mitochondrial metabolisms leading to paramylon synthesis. When grown in continuous light under pure nitrogen and in the presence of lactate as the sole carbon source, sugar synthesis occurs during the first 24 h of culture with the participation of both mitochondria (using lactate) and of chloroplasts (fixing CO$_2$ from lactate decarboxylation). The activities of ribulose bisphosphate carboxylase, phosphoenolpyruvate carboxylase, and phosphoenolpyruvate carboxykinase are very high and mitochondria and chloroplasts develop then a common network of vesicles in which paramylon grains can be seen. Electron micrographs demonstrate membrane continuity between the two types of organelles. Occasionally the mitochondrial matrix and the chloroplast stroma are separated by only a unit membrane.

**Key words:** Aerophily (micro-) – Chloroplasts – *Euglena* – Mitochondria – Paramylon synthesis.

**Introduction**

*Euglena* is a particular protist placed at the crossroads between the animal and plant kingdoms. This unicellular algae thiamine and vitamin B$_12$ dependent has a high capacity for physiological adaptation to diverse culture conditions. Photoautotrophic *Euglena* cells behave as plants and synthesize carbohydrate reserves through photosynthetically supplied energy and ribose bisphosphate (RuBP) carboxylase activity. Paramylon grains which form are external to the chloroplast, surrounded by a membrane and contiguous with the pyrenoid always present in such photoautotrophic cells (Gibbs 1962; Schiff and Epstein 1968; Orcival-Lafont and Calvayrac 1974). When grown under heterotrophic conditions, i.e., in inorganic medium supplemented with an utilisable carbon substrate and in darkness, the algae progressively lose their chlorophyll and their chloroplasts regress to form proplastids. At the beginning of exponential growth under heterotrophic conditions, dark-grown *Euglena* cells accumulate large quantities of their reserve substance, paramylon (Calvayrac and Briand 1978). Paramylon synthesis in such cells occurs in vesiculated offshoots of the chondriome (Briand and Calvayrac 1980). Lactate for example is thus very rapidly transformed to paramylon and the study of lactate metabolism demonstrated a gluconeogenetic process via a phosphoenolpyruvate (PEP) carboxykinase activity in the chondriome at the beginning of exponential growth (Briand et al. 1981). We confirmed the existence of dark CO$_2$ fixation, as shown by others (Cook 1965; Levedahl 1966), which involves a mitochondrial PEP carboxylase (Codd and Merritt 1971; Karn et al. 1973; Ohman and Ouah 1969; Wolpert and Ernst-Fonberg 1975).

At the beginning of exponential growth of photoheterotrophic *Euglena*, photosynthesis is either partially (Orcival-Lafont and Calvayrac 1974) or totally inhibited (App and Jagendorf 1963), depending on the nature of the carbon substrate present (lactate or ethanol); even though the chloroplasts are present they are then lacking pyrenoids. In the presence of lactate, cell paramylon contents are as high as they are in cells grown in darkness, implying the major role of the chondriome in paramylon biosynthesis (Calvayrac and Briand 1978; Briand and Calvayrac 1980).

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**Abbreviations:** Chl=chlorophyll; OAA=oxaloacetic acid; PEP=phosphoenolpyruvate; RuBP=ribulose bisphosphate; DTT=1,4-dithiothreitol; PVP=polyvinylpyrrolidone
This report presents special culture conditions in the light and under nitrogen atmosphere. Two strains were used, the wild strain *Euglena gracilis* Z and its DCMU-resistant variant ZR (Laval-Martin et al. 1977; Calvayrac et al. 1979a and b) which exhibits stronger heterotrophic characteristics than the wild type (Laval-Martin et al. 1981). We describe the physiological collaboration existing between mitochondria and chloroplasts during paramylon biosynthesis as well as the infrastructural interactions observable at the level of these two organelles.

**Materials and methods**

**Cells and cultures.** *Euglena gracilis* Z and ZR were grown in 21 toxin flask in 800 ml organic medium supplemented with 33 mmol 1⁻¹ lactate, pH 3.5, at 25°C in continuous light (1,500 lx) supplied by Philips daylight fluorescent tubes. A control culture of the Z strain was grown in air. The experimental cultures of strains Z and ZR were inoculated into sterile medium previously bubbled with nitrogen (L’Air liquide, grade U), sterilized by two passages through alcohol bubblers. Bubbling was continued one hour after inoculation. The culture flask was then hermetically sealed and the changes in oxygen concentrations within the system were followed with a Clark-type oxygen electrode as a function of culture age. When the sampling was performed using a syringe, a slight positive nitrogen pressure was created.

**Light requirement for lactate metabolization.** Under the culture conditions previously described, we furnished [1⁴C]lactate (specific radioactivity: 1,890 MBq mmol⁻¹; total radioactivity: 1.85 MBq) as tracer of the metabolized lactate in culture performed for 3 days under a light regimen consisting of 16 h light (1,500 lx) and 8 h darkness. The sampling of 20 ml cell culture was done at the very moment of the luminous transitions, simultaneously with a scanning by a nitrogen stream of the atmosphere present in the toxin flask. The CO₂ was then trapped in the KOH contained in a bubbler. The cell culture was centrifuged for 10 min at 2,000 g. A scintillator counter was used to determine the ¹⁴C incorporation in 100 μl aliquots of the KOH, having trapped the CO₂, and of the 2,000 g pellet resuspended in 1 ml water (cells). The aliquots were added to 5 ml Bray scintillant fluid (Bray 1960).

**Chlorophyll assay.** Chlorophyll was quantitatively determined by extraction with 80% aqueous acetone and assayed as described by Mac Kinney (1941).

**Capacities of respiratory and photosynthetic oxygen exchanges.** A Clark-type membrane electrode was used to measure respiratory oxygen consumption, as well as photosynthetic oxygen evolution in the saturating light of cells taken from cultures and assayed in the electrode chamber under normal atmosphere.

**Enzyme assays.** **Carboxylase assays by ¹⁴CO₂ incorporation.** The activities of RuBP carboxylase (EC 4.1.1.39) and PEP carboxylase (EC 4.1.1.31) were assayed as previously described (Laval-Martin et al. 1981), the protein content in extracts being determined with the method of Lowry et al. (1951).

**PEP carboxylase spectrophotometric assay.** The method described by Jomain-Baum et al. (1976) was used. This activity is measured as an increase in A₃₄₀ due to the formation of NADH + H⁺ (which accompanies oxaloacetic acid (OAA) formation from malate). Absorbance changes were totally inhibited by 100 μl mer- captopicolinic acid, a specific inhibitor of animal PEP carboxykinase (Jomain-Baum et al. 1976; Liang et al. 1976), and of plant one (Rathnam and Edwards 1977). The initial rate of the enzyme was taken from the linear portions of the curves.

**Electron microscopy.** Each cell sample was prefixed directly in the culture medium for 10 min with glutaraldehyde at a final concentration of 2%. After centrifugation, the cells were resuspended in 4% glutaraldehyde in phosphate buffer (pH 7) for one hour. Post-fixation was done with 1% osmium tetroxide in phosphate buffer (pH 7) for one hour at 0°C. Samples were embedded in Epon and sectioned with an LKB ultramicrotome. Observation was performed with a Hitachi HU 11 electron microscope.

**Results**

When *Euglena* cells are grown with lactate as sole carbon source under nitrogen atmosphere, no cell mortality was observed and cell generation time was light-intensity dependent. The light intensity of 1,500 lx was sufficient to allow generation time identical to that to the control in normal air (21% oxygen). Oxygen electrode measurements of cultures under nitrogen showed a slight enrichment in O₂. After 72 h of growth in the light the medium was 1% O₂ enriched and after 96 h the corresponding figure was 3% (in reference to 21% atmospheric oxygen). Therefore the cells were growing in micro-aerophily.

Experiments with [1⁴C]lactate indicate that the substrate was consumed during the exponential phase of culture under light with some very low evolution of CO₂ (Fig. 1). When illumination was suppressed for a certain period, lactate uptake practically stopped but CO₂ evolution remained stronger than under light. We conclude that lactate is the carbon source used for growth under light, a large part of the CO₂ then evolved being fixed due to photosynthetic activi-